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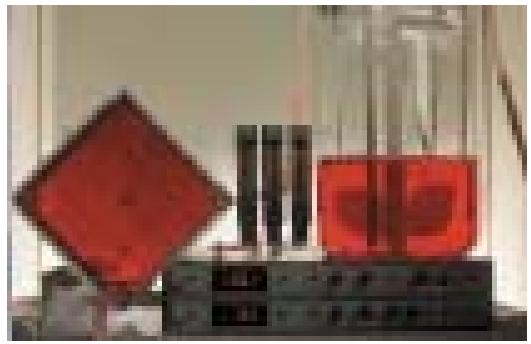

Scope & Practicality of *In Vivo* Testing for Adventitious Agents

Vaccine Cell Substrates 2004
June 30, 2004
Mary D. Whiteman
Rockville, Maryland

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Potential Dangers of Biologics

- Oncogenes
- Potential To Stimulate Oncogenes
- Bacterial, Fungal and Mycoplasmal Contamination
- Viruses - Human
- Viruses - Rodent

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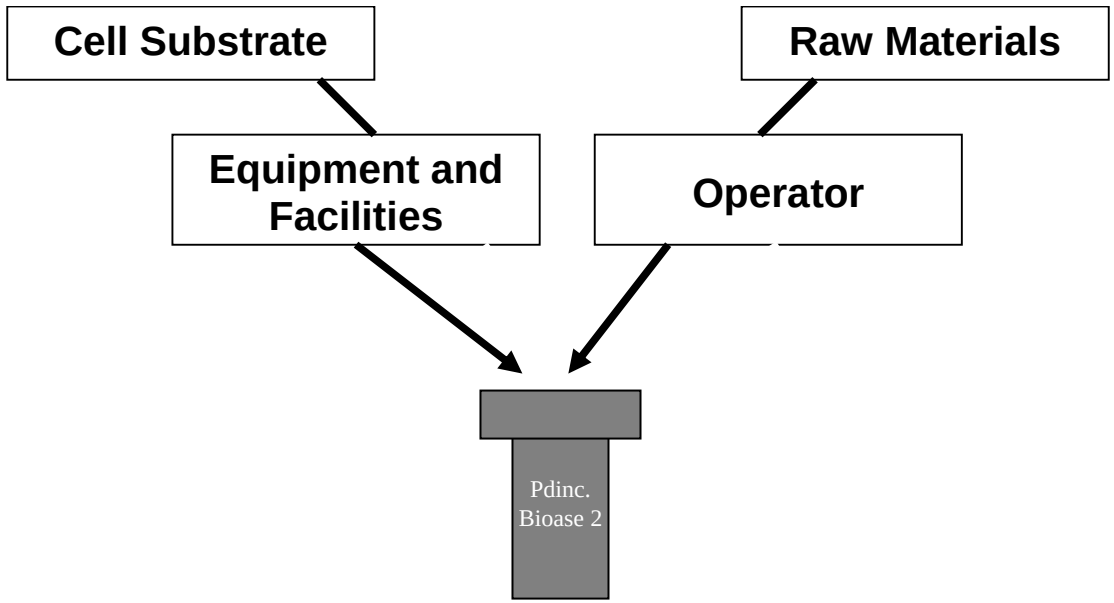
Portals of Entry for Contaminants



Safety Testing of Biologics

Key Regulatory Guidelines and Documents

- United States
  - 21 CFR Parts 58, 200-299, 600-680
  - “Points to Consider” - CBER (1997)
- European Union
  - “Notes for Guidance” - CPMP (1998)
- International Conference on Harmonization (ICH)
  - ICH Viral Safety Document (1997)

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Historical Instances of Viral Contamination of Biologics

Product	**Contaminant**
Yellow Fever Vaccine	Avian Leukosis Virus, Hepatitis B Virus
Poliovirus Vaccine	SV40
Poliovirus Vaccine	Live Poliovirus
Adenovirus Vaccine	SV40
Blood Products	HIV
Human Growth Hormone	Creutzfeldt-Jakob Disease

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Products Requiring Viral Safety Assessment

- Biopharmaceutical Products
  - Monoclonal Antibodies – Mouse, Rat, Human
  - Proteins from Genetically Engineered Mammalian Cell Lines (e.g. CHO)
- Gene Therapy Products (Viral Vectors)
- Human Blood Derived Products
- Biological Pharmaceuticals
  - Heparin (Bovine Origin)
  - Collagen

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Products Requiring Viral Safety Assessment (cont'd)

- Vaccines
- Xenotransplants
- Supplements/Raw Materials used in Pharmaceuticals
  - Bovine Serum
  - Sheep Blood
  - Peptones/Amino Acids
  - Bovine Insulin/Transferrin

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Viral Contamination of Biologics

1. Selection and testing of the source materials for the absence of virus.
2. Testing the product at appropriate stages of production for the absence of virus.
3. Testing the capacity of the production processes to remove or inactivate viruses.

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Testing of Starting Materials and Final Product Limitations

- No single test can demonstrate the presence of all known viruses.
- All test systems require a minimum level of viral contamination to record a positive.

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Probability of Detecting Low Concentrations of Virus

- If the sample contains a low concentration of virus and only a fraction of the sample is assayed, the fraction may test negative due to random and unequal distribution throughout the sample.

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In Vivo *Assay for Adventitious Viral Contaminants*

- Viruses that do not cause cytopathic or other noticeable effects in a cell culture system may be detectable in an animal system.

In Vivo *Testing Guidelines for Detecting Adventitious Contaminants in Vaccines and Biologics*

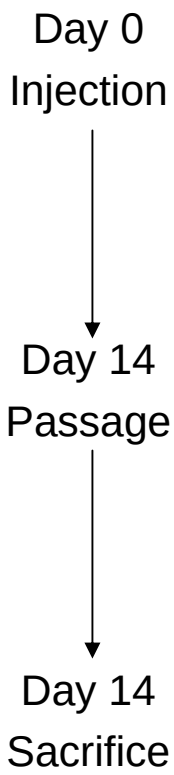
- 21 CFR Part 630.35 (Revoked)
- 21 CFR Section 630.16 (Revoked)
- Jacobs JP, McGrath DI, Garrett AJ and Schild GC. 1981. Guidelines for the acceptability, management and testing of serially propagated human diploid cells for the production of live virus vaccine for use in man. *J. Biol. Stand.,* **9**, 331-342
- European Pharmacopoeia Commission 1999. Tests for Extraneous Agents in Viral Vaccines for Human Use (General method N^{o} 2.6.16.). European Pharmacopoeia 3rd edition Supplement: 50-51.

In Vivo *Assays for Adventitious Contaminants*

- Animal systems
  - Suckling mice
  - Adult mice
  - Guinea pigs
  - Embryonated hen’s eggs

In Vivo *Assays for Adventitious Contaminants*

- **Experimental Design – Suckling Mice**



- Each pup injected
  - intraperitoneally (i.p.)
  - intracranially (i.c.)
  - per os (p.o.)
- Animals observed for 14 days.
- On day 14, a single pool of emulsified tissue of all surviving mice is prepared and passaged.
- Passage animals observed for 14 days.

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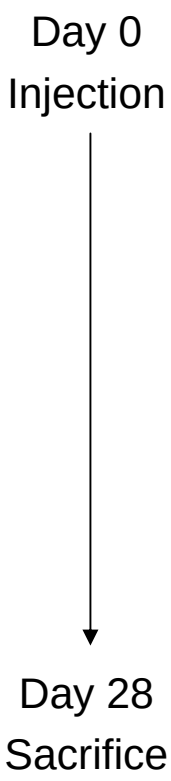
Isolation and Detection of Viruses – Suckling Mice

- **Agents for which suckling mice are an efficient isolation system:**
  - Arboviruses
  - Coxsackie A viruses
  - Coxsackie B viruses
  - Herpes Simplex (type 1 and 2)
  - Rhabdoviruses (including rabies)
  - Togaviruses (LDV)
  - Junin
  - Herpes B
- **Agents for which suckling mice are of secondary efficiency as an isolation system:**
  - LCM
  - Lassa
  - Hantaan
  - Ebola
  - Vaccinia

In Vivo *Assays for Adventitious Contaminants*

- **Experimental Design – Adult Mice**



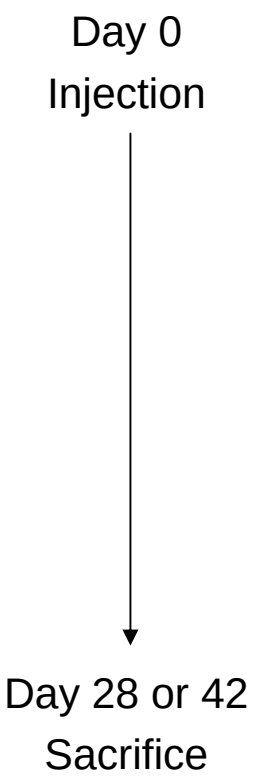
- Each mouse injected
  - intraperitoneally (i.p.)
  - intracranially (i.c.)
  - per os (p.o.)
  - intranasally (i.n.)
- Animals observed for 28 days.

Isolation and Detection of Viruses – Adult Mice

- **Agents for which adult mice are an efficient isolation system:**
  - Rhabdoviruses (including rabies)
  - Togaviruses (LDV)
  - LCM
- **Agents for which adult mice are of secondary efficiency as an isolation system:**
  - Arboviruses
  - Herpes Simplex (type 1 and 2)
  - Lassa

In Vivo *Assays for Adventitious Contaminants*

- **Experimental Design – Guinea pigs**



- Each guinea pig injected
  - intraperitoneally (i.p.)
  - intracranially (i.c.)
- Injection sites observed weekly for lesions.
- Animals observed for at least 28 days.
- For vaccine tests, animals observed for 42 days.

Isolation and Detection of Viruses – Guinea pigs

- **Agents for which guinea pigs are an efficient isolation system:**
  - Rhabdoviruses (including rabies)
  - LCM
  - Lassa
  - Junin
  - Marburg
  - Ebola
- **Agents for which guinea pigs are of secondary efficiency as an isolation system:**
  - Arboviruses
  - Vaccinia

In Vivo *Assays for Adventitious Contaminants*

- **Experimental Design – Allantoic Route**

Day 0
Injection

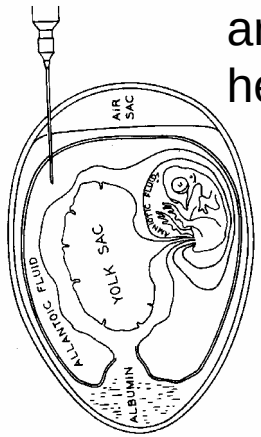
↓

Day 3
Viability and Hemagglutination Test
Passage

↓

Day 3
Viability and Hemagglutination Test

- Eggs injected and incubated for 3 days.
- Allantoic fluids are tested for hemagglutinins.
- Fluids are pooled and passaged.
- Fluids from passage eggs are tested for hemagglutinins.



In Vivo *Assays for Adventitious Contaminants*

- **Experimental Design – Yolk Sac Route**

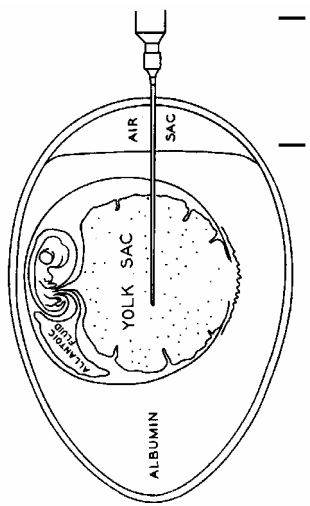
Day 0
Injection

↓

Day 9
Viability
Passage

↓

Day 9
Viability



- Each egg injected and incubated for at least 9 days.
- Examined for viability.
- Yolk sac material is pooled and passaged.
- Eggs incubated for at least 9 days.
- Examined for viability.

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Isolation and Detection of Viruses – Embryonated Hen's Eggs

- **Agents for which hen's eggs are an efficient isolation system:**
  - Herpes Simplex (type1 and 2)
  - Rhabdoviruses (including rabies)
  - Herpes B
  - Mumps
  - Influenza
  - Parainfluenza (types 1, 2 and 3)
  - Vaccinia
- **Agents for which hen's eggs are of secondary efficiency as an isolation system:**
  - Arboviruses

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In Vivo *Assays for Adventitious Contaminants*

- Evaluation of Test Results
  - The test material will be considered not contaminated with adventitious viral contaminants if the following are met:
    - 80% of animals/eggs
      - Remain healthy
      - Survive the observation period
      - No evidence of viral infection

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Assay Problems Unrelated to Test Materials

- Suckling mice
  - Death by natural causes
  - Runting
  - Inadequate maternal care
  - Cannibalization
  - Injection trauma
- Adult Mice/Guinea pigs
  - Death by natural causes/injection trauma
  - Neurological clinical signs due to i.c. injection
- Eggs
  - Hardiness (death with no assignable cause)
  - Bacterial contamination

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Assay Problems Related to Test Materials

- Gene Therapy Vectors
  - Toxicity
  - Infection
  - Hemagglutination
- Bulk Harvest Material
  - Media/Component toxicity
- Vaccine Viral Stocks and/or substrates
  - Substrate bioburden
  - Virus infects test system
  - Virus hemagglutinates
  - Viral neurovirulence
  - Antiserum toxicity

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Control of Test Material Related Problems

- Perform feasibility and/or qualification studies
  - Dilution
  - Neutralization
  - Antibiotics
  - Antiserum concentration
  - Antiserum control animals
  - Elimination of one or more test systems or routes of injection
  - Determination of viral breakthrough

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Trend Analysis

Data from assays performed at Rockville, MD from
March 28, 2003 through August 31, 2003

Test System	Number of Assays Examined	Number of System Suitability Problems	System Suitability
Suckling Mice	98	7	93%
Adult Mice	98	1	99%
Guinea pigs	88	2	98%
Allantoic route	100	3	97%
Yolk sac route	100	5	95%

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In Vivo *Testing Assays*

- Tests for Adventitious Contaminants
- Antibody Production Tests
- Tumorigenicity Test
- Rabbit Pyrogen Tests (USP and EP compliant)
- General Safety Tests (USP and EP compliant)
- TSE Clearance Bioassay

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